Technische Universiteit
Eindhoven
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**iGEM TU/e 2017**
Biomedical Engineering

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# LIU PCR

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LUI PCR

# 1 LIU PCR

**Estimated bench time:** 30 minutes
**Estimated total time:** 5 hours
**Purpose:** insertion of amino acids in the DNA sequence with the primers.

It is essential to work with gloves at all times to protect the DNA from DNase activity.

## 1.1 Materials

- Autoclaved $H_2O$
- Autoclaved PCR tubes
- Bucket with ice
- DNA polymerase
- dNTPs
- Forward primer
- Pipettes and tips
- Reverse primer
- Thermal cycler
- Vector DNA in which amino acids need to be inserted

## 1.2 Setup & Protocol

- Construct a PCR mixture in the following way. Start with the component with the largest volume and end with the Master Mix. Keep the Master Mix on ice.

| Component | Quantity/mass/final concentration | Volume (µl) |
|---|---|---|
| **$H_2O$** | Fill up to 50 µL | |
| **DNA** | 110 ng/µL | |
| **Primer FW** | 1 µM (10 µM stock) | 5 |
| **Primer RV** | 1 µM (10 µM stock) | 5 |
| **Q5 Mastermix (2X)** | 1X | 25 |
| ***Total*** | | 50 |

- Mix well by pipetting up and down.

| Primer | Tm (NO) | Tm (PP) | Concentration |
|---|---|---|---|
| **RV_5AA_streptagCT33** | 52 | 41 | |
| **FW_5AA_streptagCT33** | 46 | 41 | |
| **Thus for 5AA** | **44** | **35** | |
| **RV_1AA_streptagCT33** | 52 | 39-41 | |
| **FW_1AA_streptagCT33** | 56 | 39-41 | |
| **Thus for 1AA** | **48** | **35** | |

NO=primer sequence matched to the template
PP=Primer-Primer overlap

- Run the following PCR program

| Cycle | Temperature | Time | Description |
|---|---|---|---|
| **1** | 95 | 5 minutes | Denature the template DNA |
| **30x amplification** | 95 | 1 minute | |
| | **Tm(NO)-5** | 1 minute | |
| | 72 | 10-15 minutes | Depending on the length of the template constructs |
| **PCR finishing cycles** | **Tm(PP)-5** | 1 minute | Annealing step |
| | 72 | 30 minutes | Extension step |

- Digestion of PCR product with DpnI. See the protocol Digestion for more information.